That Which is Claimed is:

1. A method of synthesizing an insulin polypeptide-oligomer conjugate comprising:

contacting a proinsulin polypeptide comprising an insulin polypeptide coupled to one or more peptides by peptide bond(s) capable of being cleaved to yield the insulin polypeptide with an oligomer comprising a hydrophilic moiety and a lipophilic moiety under conditions sufficient to couple the oligomer to the insulin polypeptide portion of the proinsulin polypeptide and provide a proinsulin polypeptide-oligomer conjugate; and

cleaving the one or more peptides from the proinsulin polypeptide-oligomer conjugate to provide the insulin polypeptide-oligomer conjugate.

2. The method according to Claim 1, wherein the contacting of the proinsulin polypeptide with the oligomer comprises:

contacting the oligomer with an activating agent under conditions sufficient to provide an activated oligomer capable of coupling to a nucleophilic functionality on the proinsulin polypeptide; and

contacting the activated oligomer with the proinsulin polypeptide under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

3. The method according to Claim 2, wherein the contacting of the oligomer with the activating agent and the contacting of the activated oligomer with the proinsulin polypeptide is performed in situ.

4. The method according to Claim 2, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 1:1.

5. The method according to Claim 2, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 3:1.

6. The method according to Claim 2, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 4:1.

7. The method according to Claim 6, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

8. The method according to Claim 6, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

9. The method according to Claim 6, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

10. The method according to Claim 6, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

11. The method according to Claim 1, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

12. The method according to Claim 1, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

13. The method according to Claim 1, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

14. The method according to Claim 1, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

15. The method according to Claim 1, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

16. The method according to Claim 1, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

17. The method according to Claim 16, wherein the connecting peptide is a C-peptide polypeptide.

18. The method according to Claim 16, wherein the connecting peptide is C-peptide.

19. The method according to Claim 16, wherein the connecting peptide is devoid of lysine residues.

20. The method according to Claim 16, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

21. The method according to Claim 20, wherein the leader peptide is devoid of lysine residues.

22. The method according to Claim 1, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides is a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and at a second end to the N-terminus of the A-chain polypeptide.

23. The method according to Claim 1, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides is a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and at a second end to the N-terminus of the A-chain polypeptide, and a leader peptide coupled to the N-terminus of the B-chain polypeptide.

24. The method according to Claim 1, wherein the proinsulin polypeptide is proinsulin.

25. The method according to Claim 1, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable.

26. The method according to Claim 1, wherein the insulin polypeptide is insulin.

27. The method according to Claim 26, wherein the oligomer is coupled to the lysine at the B29 position of the insulin.

28. The method according to Claim 1, wherein the insulin polypeptide is an insulin analog selected from the group consisting of Gly^{A21} insulin, human; Gly^{A21} Gln^{B3} insulin, human; Ala^{A21} insulin, human; Ala^{A21} Gln^{B3} insulin, human; Gln^{B3} insulin, human; Gln^{B30} insulin, human; Gly^{A21} Glu^{B30} insulin, human; Gly^{A21} Gln^{B3} Glu^{B30} insulin, human; Gln^{B3} Glu^{B30} insulin, human; Asp^{B28} insulin, human; Lys^{B28} insulin, human; Leu^{B28} insulin, human; Val^{B28} insulin, human; Ala^{B28} insulin, human; Asp^{B28} Pro^{B29} insulin, human; Lys^{B28} Pro^{B29} insulin, human; Leu^{B28} Pro^{B29} insulin, human; Val^{B28} Pro^{B29} insulin, human; Ala^{B28} Pro^{B29} insulin, human.

29. The method according to Claim 1, wherein the insulin polypeptide-oligomer conjugate is amphiphilically balanced.

30. The method according to Claim 1, wherein the oligomer is present as a substantially monodispersed mixture.

31. The method according to Claim 1, wherein the oligomer is present as a monodispersed mixture.

32. The method according to Claim 1, wherein the hydrophilic moiety is a polyalkylene glycol moiety.

33. The method according to Claim 32, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

34. The method according to Claim 32, wherein the polyalkylene glycol moiety has between 1 and 50 polyalkylene glycol subunits.

35. The method according to Claim 32, wherein the polyalkylene glycol moiety has between 3 and 50 polyalkylene glycol subunits.

36. The method according to Claim 32, wherein the polyalkylene glycol moiety has between 2 and 10 polyalkylene glycol subunits.

37. The method according to Claim 32, wherein the polyalkylene glycol moiety has between 4 and 10 polyalkylene glycol subunits.

38. The method according to Claim 32, wherein the polyalkylene glycol moiety has at least 2 polyalkylene glycol subunits.

39. The method according to Claim 1, wherein the lipophilic moiety is an alkyl or fatty acid moiety.

40. The method according to Claim 1, wherein the lipophilic moiety has between 1 and 28 carbon atoms.

41. The method according to Claim 1, wherein the lipophilic moiety has between 2 and 24 carbon atoms.

42. The method according to Claim 1, wherein the lipophilic moiety has between 3 and 18 carbon atoms.

43. The method according to Claim 1, wherein the lipophilic moiety has between 4 and 12 carbon atoms.

44. The method according to Claim 1, wherein the lipophilic moiety has between 5 and 7 carbon atoms.

45. The method according to Claim 1, wherein the lipophilic moiety has between 4 and 14 carbon atoms.

46. The method according to Claim 1, wherein the cleaving of the one or more peptides from the proinsulin polypeptide-oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under

conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-oligomer conjugate.

47. The method according to Claim 46, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

48. The method according to Claim 16, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the cleaving of the connecting peptide from the proinsulin polypeptide-oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin polypeptide-oligomer conjugate.

49. The method according to Claim 48, wherein the terminal amino acid residue is an arginine residue.

50. The method according to Claim 49, wherein the insulin polypeptide is insulin, and wherein the connecting peptide is human C-peptide.

51. The method according to Claim 48, wherein the contacting of the proinsulin polypeptide-oligomer conjugate with a first enzyme and the contacting of the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme occur substantially concurrently.

52. The method according to Claim 51, wherein the first enzyme and the second enzyme are provided in a mixture comprising the first enzyme and the second enzyme.

53. The method according to Claim 48, wherein the first enzyme is trypsin, and wherein the second enzyme is carboxy peptidase B.

54. A method of synthesizing an insulin polypeptide-oligomer conjugate comprising:

contacting a proinsulin polypeptide comprising an insulin polypeptide coupled to one or more peptides by peptide bond(s) capable of being cleaved to yield the insulin polypeptide with an oligomer comprising the structure of Formula I:

$$A\text{——}L_j\text{——}G_k\text{——}R\text{——}G'_m\text{——}R'\text{——}G''_n\text{——}T \qquad (I)$$

wherein:

A is an activatable moiety;

L is a linker moiety;

G, G' and G" are each spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and

j, k, m and n are individually 0 or 1;

under conditions sufficient to couple the oligomer to the insulin polypeptide portion of the proinsulin polypeptide and provide a proinsulin polypeptide-oligomer conjugate; and

cleaving the one or more peptides from the proinsulin polypeptide-oligomer conjugate to provide the insulin polypeptide-oligomer conjugate.

55. The method according to Claim 54, wherein A is selected from the group consisting of -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, and NH_2.

56. The method according to Claim 54, wherein L is selected from the group consisting of alkyl moieties and fatty acid moieties.

57. The method according to Claim 54, wherein G, G' and G" are individually selected from the group consisting of sugar moieties, cholesterol, and glycerine moieties.

58. The method according to Claim 54, wherein T is selected from the group consisting of alkyl and alkoxy.

59. The method according to Claim 54, wherein the contacting of the proinsulin polypeptide to the oligomer comprises:

contacting the oligomer with an activating agent under conditions sufficient to provide an activated oligomer capable of coupling to a nucleophilic functionality on the proinsulin polypeptide; and

contacting the activated oligomer with the proinsulin polypeptide under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

60. The method according to Claim 59, wherein the contacting of the oligomer with the activating agent and the contacting of the activated oligomer with the proinsulin polypeptide is performed in situ.

61. The method according to Claim 59, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 1:1.

62. The method according to Claim 59, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 3:1.

63. The method according to Claim 59, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 4:1.

64. The method according to Claim 63, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

65. The method according to Claim 63, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

66. The method according to Claim 63, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

67. The method according to Claim 63, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

68. The method according to Claim 63, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

69. The method according to Claim 54, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

70. The method according to Claim 54, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

71. The method according to Claim 54, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

72. The method according to Claim 54, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

73. The method according to Claim 54, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

74. The method according to Claim 54, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

75. The method according to Claim 74, wherein the connecting peptide is a C-peptide polypeptide.

76. The method according to Claim 74, wherein the connecting peptide is C-peptide.

77. The method according to Claim 74, wherein the connecting peptide is devoid of lysine residues.

78. The method according to Claim 74, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

79. The method according to Claim 74, wherein the leader peptide is devoid of lysine residues.

80. The method according to Claim 54, wherein the proinsulin polypeptide is proinsulin.

81. The method according to Claim 54, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable.

82. The method according to Claim 54, wherein the insulin polypeptide is insulin.

83. The method according to Claim 82, wherein the oligomer is coupled to the lysine at the B29 position of the insulin.

84. The method according to Claim 54, wherein the insulin polypeptide-oligomer conjugate is amphiphilically balanced.

85. The method according to Claim 54, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

86. The method according to Claim 85, wherein the polyethylene glycol moiety has between 1 and 50 polyethylene glycol subunits.

87. The method according to Claim 85, wherein the polyethylene glycol moiety has between 3 and 50 polyethylene glycol subunits.

88. The method according to Claim 85, wherein the polyethylene glycol moiety has between 2 and 10 polyethylene glycol subunits.

89. The method according to Claim 85, wherein the polyethylene glycol moiety has between 4 and 10 polyethylene glycol subunits.

90. The method according to Claim 85, wherein the polyethylene glycol moiety has at least 2 polyethylene glycol subunits.

91. The method according to Claim 54, wherein the lipophilic moiety is an alkyl or a fatty acid moiety.

92. The method according to Claim 91, wherein the lipophilic moiety has between 1 and 28 carbon atoms.

93. The method according to Claim 91, wherein the lipophilic moiety has between 2 and 24 carbon atoms.

94. The method according to Claim 91, wherein the lipophilic moiety has between 3 and 18 carbon atoms.

95. The method according to Claim 91, wherein the lipophilic moiety has between 4 and 12 carbon atoms.

96. The method according to Claim 91, wherein the lipophilic moiety has between 5 and 7 carbon atoms.

97. The method according to Claim 91, wherein the lipophilic moiety has between 4 and 14 carbon atoms.

98. The method according to Claim 54, wherein:

A is a carboxylic acid moiety;

R is an alkyl moiety having between 3 and 8 carbon atoms;

R' is polyethylene glycol having between 4 and 10 polyethylene glycol subunits;

T is lower alkyl or lower alkoxy; and

j, k, m and n are 0.

99. The method according to Claim 54, wherein:

A is a carboxylic acid moiety;

R is an alkyl moiety having between 3 and 8 carbon atoms;

R' is polyethylene glycol having 7 polyethylene glycol subunits;

T is methoxy; and

j, k, m and n are 0.

100. The method according to Claim 54, wherein the cleaving of the one or more peptides from the proinsulin polypeptide-oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-oligomer conjugate.

101. The method according to Claim 100, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

102. The method according to Claim 74, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the cleaving of the connecting peptide from the proinsulin-oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin-oligomer conjugate.

103. The method according to Claim 102, wherein the terminal amino acid residue is an arginine residue.

104. The method according to Claim 103, wherein the insulin polypeptide is insulin, and wherein the connecting peptide is human C-peptide.

105. The method according to Claim 102, wherein the contacting of the proinsulin-oligomer conjugate with a first enzyme and the contacting of the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme occur substantially concurrently.

106. The method according to Claim 105, wherein the first enzyme and the second enzyme are provided in a mixture comprising the first enzyme and the second enzyme.

107. The method according to Claim 102, wherein the first enzyme is trypsin, and wherein the second enzyme is carboxy peptidase B.

108. A method of synthesizing an insulin polypeptide-oligomer conjugate comprising:

contacting a proinsulin polypeptide comprising an insulin polypeptide coupled to one or more peptides by peptide bond(s) capable of being cleaved to yield the insulin polypeptide with an oligomer comprising the structure of Formula I:

$$A\text{——}L_j\text{——}G_k\text{——}R\text{——}G'_m\text{——}R'\text{——}G''_n\text{——}T \qquad (I)$$

wherein:

A is an activatable moiety selected from the group consisting of -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, and NH_2;

L is a linker moiety selected from the group consisting of alkyl moieties and fatty acid moieties;

G, G' and G" are each spacer moieties individually selected from the group consisting of sugar moieties, cholesterol, and glycerine moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety selected from the group consisting of alkyl and alkoxy; and

j, k, m and n are individually 0 or 1;

under conditions sufficient to couple the oligomer to the insulin polypeptide portion of the proinsulin polypeptide and provide a proinsulin polypeptide-oligomer conjugate; and

cleaving the one or more peptides from the proinsulin polypeptide-oligomer conjugate to provide the insulin polypeptide-oligomer conjugate.

109. A method of synthesizing an insulin polypeptide-oligomer conjugate comprising:

contacting a proinsulin polypeptide comprising an insulin polypeptide coupled to one or more peptides by peptide bond(s) capable of being cleaved to yield the insulin polypeptide with an oligomer comprising the structure of Formula II:

$$A\text{——}X(CH_2)_mY(C_2H_4O)_nR \qquad (II)$$

wherein:

A is -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, or NH_2;

X is an oxygen atom or a covalent bond, with the proviso that X is not an oxygen atom when A is -OH;

Y is an ester, an ether, a carbamate, a carbonate, or an amide bonding moiety;

m is between 1 and 30;

n is between 1 and 50; and

R is an alkyl moiety, a sugar moiety, cholesterol, adamantane, an alcohol moiety, or a fatty acid moiety;

under conditions sufficient to couple the oligomer to the insulin polypeptide portion of the proinsulin polypeptide and provide a proinsulin polypeptide-oligomer conjugate; and

cleaving the one or more peptides from the proinsulin polypeptide-oligomer conjugate to provide the insulin polypeptide-oligomer conjugate.

110. The method according to Claim 109, wherein the contacting of the proinsulin polypeptide to the oligomer comprises:

contacting the oligomer with an activating agent under conditions sufficient to provide an activated oligomer capable of coupling to a nucleophilic functionality on the proinsulin polypeptide; and

contacting the activated oligomer with the proinsulin polypeptide under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

111. The method according to Claim 110, wherein the contacting of the oligomer with the activating agent and the contacting of the activated oligomer with the proinsulin polypeptide is performed in situ.

112. The method according to Claim 110, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 1:1.

113. The method according to Claim 110, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 3:1.

114. The method according to Claim 110, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 4:1.

115. The method according to Claim 114, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

116. The method according to Claim 114, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

117. The method according to Claim 114, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

118. The method according to Claim 114, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

119. The method according to Claim 114, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

120. The method according to Claim 109, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

121. The method according to Claim 109, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

122. The method according to Claim 109, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

123. The method according to Claim 109, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

124. The method according to Claim 109, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

125. The method according to Claim 109, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

126. The method according to Claim 125, wherein the connecting peptide is a C-peptide polypeptide.

127. The method according to Claim 125, wherein the connecting peptide is C-peptide.

128. The method according to Claim 125, wherein the connecting peptide is devoid of lysine residues.

129. The method according to Claim 125, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

130. The method according to Claim 129, wherein the leader peptide is devoid of lysine residues.

131. The method according to Claim 109, wherein the proinsulin polypeptide is proinsulin.

132. The method according to Claim 109, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable.

133. The method according to Claim 109, wherein the insulin polypeptide is insulin.

134. The method according to Claim 133, wherein the oligomer is coupled to the lysine at the B29 position of the insulin.

135. The method according to Claim 109, wherein the insulin polypeptide-oligomer conjugate is amphiphilically balanced.

136. The method according to Claim 109, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

137. The method according to Claim 109, wherein m is between 3 and 16.

138. The method according to Claim 109, wherein m is between 4 and 14.

139. The method according to Claim 109, wherein m is between 5 and 10.

140. The method according to Claim 109, wherein n is between 3 and 18.

141. The method according to Claim 109, wherein n is between 4 and 14.

142. The method according to Claim 109, wherein n is between 5 and 10.

143. The method according to Claim 109, wherein R is lower alkyl.

144. The method according to Claim 109, wherein R is C_1 to C_3 alkyl.

145. The method according to Claim 109, wherein R is methyl.

146. The method according to Claim 109, wherein the cleaving of the one or more peptides from the proinsulin polypeptide-oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-oligomer conjugate.

147. The method according to Claim 146, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

148. The method according to Claim 125, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the cleaving of the connecting peptide from the proinsulin-oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin-oligomer conjugate.

149. The method according to Claim 148, wherein the terminal amino acid residue is an arginine residue.

150. The method according to Claim 149, wherein the insulin polypeptide is insulin, and wherein the connecting peptide is human C-peptide.

151. The method according to Claim 148, wherein the contacting of the proinsulin-oligomer conjugate with a first enzyme and the contacting of the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme occur substantially concurrently.

152. The method according to Claim 151, wherein the first enzyme and the second enzyme are provided in a mixture comprising the first enzyme and the second enzyme.

153. The method according to Claim 148, wherein the first enzyme is trypsin, and wherein the second enzyme is carboxy peptidase B.

154. A method of synthesizing an insulin polypeptide-oligomer conjugate comprising:

contacting a proinsulin polypeptide comprising an insulin polypeptide coupled to one or more peptides by peptide bond(s) capable of being cleaved to yield the insulin polypeptide with an oligomer comprising the structure of Formula III:

$$A\text{-}(CH_2)_m(OC_2H_4)_nOR \qquad (III)$$

wherein:

A is -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, or NH_2;

m is between 1 and 25;

n is between 1 and 25; and

R is alkyl;

under conditions sufficient to couple the oligomer to the insulin polypeptide portion of the proinsulin polypeptide and provide a proinsulin polypeptide-oligomer conjugate; and

cleaving the one or more peptides from the proinsulin polypeptide-oligomer conjugate to provide the insulin polypeptide-oligomer conjugate.

155. The method according to Claim 154, wherein the contacting of the proinsulin polypeptide with the oligomer comprises:

contacting the oligomer with an activating agent under conditions sufficient to provide an activated oligomer capable of coupling to a nucleophilic functionality on the proinsulin polypeptide; and

contacting the activated oligomer with the proinsulin polypeptide under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

156. The method according to Claim 155, wherein the contacting of the oligomer with the activating agent and the contacting of the activated oligomer with the proinsulin polypeptide is performed in situ.

157. The method according to Claim 155, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 1:1.

158. The method according to Claim 155, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 3:1.

159. The method according to Claim 155, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 4:1.

160. The method according to Claim 159, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

161. The method according to Claim 159, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

162. The method according to Claim 159, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

163. The method according to Claim 159, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

164. The method according to Claim 159, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

165. The method according to Claim 154, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

166. The method according to Claim 154, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

167. The method according to Claim 154, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

168. The method according to Claim 154, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

169. The method according to Claim 154, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

170. The method according to Claim 154, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

171. The method according to Claim 170, wherein the connecting peptide is a C-peptide polypeptide.

172. The method according to Claim 170, wherein the connecting peptide is C-peptide.

173. The method according to Claim 170, wherein the connecting peptide is devoid of lysine residues.

174. The method according to Claim 170, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

175. The method according to Claim 170, wherein the leader peptide is devoid of lysine residues.

176. The method according to Claim 154, wherein the proinsulin polypeptide is proinsulin.

177. The method according to Claim 154, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable .

178. The method according to Claim 154, wherein the insulin polypeptide is insulin.

179. The method according to Claim 178, wherein the oligomer is coupled to the lysine at the B29 position of the insulin.

180. The method according to Claim 154, wherein the insulin polypeptide-oligomer conjugate is amphiphilically balanced.

181. The method according to Claim 154, wherein m is between 3 and 16.

182. The method according to Claim 154, wherein m is between 4 and 14.

183. The method according to Claim 154, wherein m is between 5 and 10.

184. The method according to Claim 154, wherein n is between 3 and 18.

185. The method according to Claim 154, wherein n is between 4 and 14.

186. The method according to Claim 154, wherein n is between 5 and 10.

187. The method according to Claim 154, wherein R is lower alkyl.

188. The method according to Claim 154, wherein R is C_1 to C_3 alkyl.

189. The method according to Claim 154, wherein R is methyl.

190. The method according to Claim 154, wherein the cleaving of the one or more peptides from the proinsulin polypeptide-oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-oligomer conjugate.

191. The method according to Claim 190, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

192. The method according to Claim 170, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the cleaving of the connecting peptide from the proinsulin-oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin-oligomer conjugate.

193. The method according to Claim 192, wherein the terminal amino acid residue is an arginine residue.

194. The method according to Claim 193, wherein the insulin polypeptide is insulin, and wherein the connecting peptide is human C-peptide.

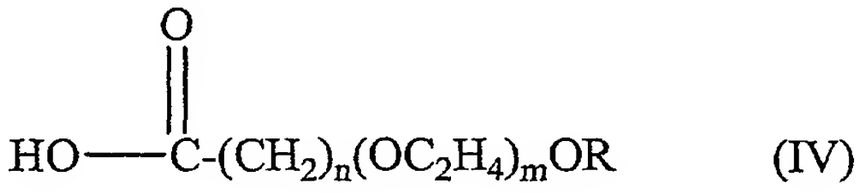
195. The method according to Claim 192, wherein the contacting of the proinsulin-oligomer conjugate with a first enzyme and the contacting of the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme occur concurrently.

196. The method according to Claim 195, wherein the first enzyme and the second enzyme are provided in a mixture comprising the first enzyme and the second enzyme.

197. The method according to Claim 192, wherein the first enzyme is trypsin, and wherein the second enzyme is carboxy peptidase B.

198. A method of synthesizing an insulin polypeptide-oligomer conjugate comprising:

contacting a proinsulin polypeptide comprising an insulin polypeptide having an A-chain polypeptide and a B-chain polypeptide, which comprises a lysine residue; a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide; and a leader peptide coupled to the N-terminus of the B-chain polypeptide with an oligomer comprising the structure of Formula IV:



(IV)

wherein:

n is between 1 and 30;

m is between 1 and 50; and

R is alkyl;

under conditions sufficient to couple the oligomer to the lysine residue of the B-chain polypeptide of the insulin polypeptide portion of the proinsulin polypeptide and provide a proinsulin polypeptide-oligomer conjugate; and

enzymatically cleaving the connecting peptide and the leader peptide from the proinsulin polypeptide-oligomer conjugate to provide the insulin polypeptide-oligomer conjugate.

199. The method according to Claim 198, wherein the A-chain polypeptide is devoid of lysine residues, wherein the B-chain polypeptide comprises a single lysine residue, and wherein the enzymatically cleaving of the connecting peptide and the leader peptide from the proinsulin polypeptide-oligomer conjugate provides an insulin polypeptide-oligomer conjugate product consisting of an insulin polypeptide-oligomer mono-conjugate having an oligomer coupled to the lysine residue.

200. The method according to Claim 199, wherein the contacting of the proinsulin polypeptide with the oligomer comprises:

contacting the oligomer with an activating agent under conditions sufficient to provide an activated oligomer capable of coupling to a nucleophilic functionality on the proinsulin polypeptide; and

contacting the activated oligomer with the proinsulin polypeptide under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

201. The method according to Claim 200, wherein the contacting of the oligomer with the activating agent and the contacting of the activated oligomer with the proinsulin polypeptide is performed in situ.

202. The method according to Claim 200, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 1:1.

203. The method according to Claim 200, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 3:1.

204. The method according to Claim 200, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 4:1.

205. The method according to Claim 204, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

206. The method according to Claim 204, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

207. The method according to Claim 204, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

208. The method according to Claim 204, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

209. The method according to Claim 204, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

210. The method according to Claim 198, wherein the connecting peptide is devoid of lysine residues.

211. The method according to Claim 198, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the enzymatically cleaving of the connecting peptide and the leader peptide from the proinsulin polypeptide-oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin polypeptide-oligomer conjugate.

212. The method according to Claim 211, wherein the terminal amino acid residue is arginine.

213. The method according to Claim 198, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

214. The method according to Claim 198, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

215. The method according to Claim 198, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

216. The method according to Claim 198, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

217. The method according to Claim 198, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

218. The method according to Claim 198, wherein m is between 3 and 16.

219. The method according to Claim 198, wherein m is between 4 and 14.

220. The method according to Claim 198, wherein m is between 5 and 10.

221. The method according to Claim 198, wherein n is between 3 and 18.

222. The method according to Claim 198, wherein n is between 4 and 14.

223. The method according to Claim 198, wherein n is between 5 and 10.

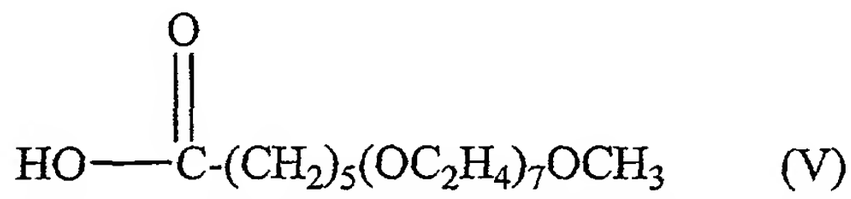
224. The method according to Claim 198, wherein R is lower alkyl.

225. The method according to Claim 198, wherein R is C_1 to C_3 alkyl.

226. The method according to Claim 198, wherein R is methyl.

227. A method of synthesizing an insulin-oligomer conjugate comprising:

contacting a proinsulin polypeptide, which comprises proinsulin coupled at its N-terminus to a leader peptide, with an oligomer comprising the structure of Formula V:



(V)

under conditions sufficient to couple the oligomer to the B29 lysine residue of the proinsulin and provide a proinsulin polypeptide-oligomer conjugate; and

enzymatically cleaving the C-peptide and the leader peptide from the proinsulin polypeptide-oligomer conjugate to provide the insulin-oligomer conjugate.

228. The method according to Claim 227, wherein the contacting of the proinsulin polypeptide with the oligomer comprises:

contacting the oligomer with an activating agent under conditions sufficient to provide an activated oligomer capable of coupling to the B29 lysine residue on the proinsulin polypeptide; and

contacting the activated oligomer with the proinsulin polypeptide under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

229. The method according to Claim 228, wherein the contacting of the oligomer with the activating agent and the contacting of the activated oligomer with the proinsulin polypeptide is performed in situ.

230. The method according to Claim 228, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 1:1.

231. The method according to Claim 228, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 3:1.

232. The method according to Claim 228, wherein the molar ratio of activated oligomer to proinsulin polypeptide is greater than about 4:1.

233. The method according to Claim 232, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

234. The method according to Claim 232, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

235. The method according to Claim 232, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

236. The method according to Claim 232, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

237. The method according to Claim 232, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

238. The method according to Claim 227, wherein the leader peptide is devoid of lysine residues.

239. The method according to Claim 227, wherein the proinsulin is human proinsulin.

240. The method according to Claim 227, wherein the enzymatically cleaving of the C-peptide and the leader peptide from the proinsulin polypeptide-oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a (Arg^{31})-insulin-oligomer conjugate; and

contacting the (Arg^{31})-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin polypeptide-oligomer conjugate.

241. The method according to Claim 240, wherein the first enzyme is trypsin.

242. The method according to Claim 240, wherein the second enzyme is carboxy peptidase B.

243. The method according to Claim 227, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 75 percent.

244. The method according to Claim 227, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 80 percent.

245. The method according to Claim 227, wherein the yield of insulin polypeptide-oligomer conjugate is greater than 85 percent.

246. The method according to Claim 227, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 90 percent.

247. The method according to Claim 227, wherein the yield of insulin polypeptide-oligomer conjugate is greater than about 95 percent.

248. A method of synthesizing an insulin polypeptide-acyl oligomer conjugate comprising enzymatically cleaving one or more peptides from a proinsulin polypeptide-acyl oligomer conjugate to provide the insulin polypeptide-acyl oligomer conjugate.

249. The method according to Claim 248, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

250. The method according to Claim 249, wherein the connecting peptide is a C-peptide polypeptide.

251. The method according to Claim 249, wherein the connecting peptide is C-peptide.

252. The method according to Claim 249, wherein the connecting peptide is devoid of lysine residues.

253. The method according to Claim 249, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

254. The method according to Claim 249, wherein the leader peptide is devoid of lysine residues.

255. The method according to Claim 248, wherein the proinsulin polypeptide is proinsulin.

256. The method according to Claim 248, wherein the proinsulin polypeptide is proinsulin coupled at its N-terminus to a leader peptide by a peptide bond that is cleavable.

257. The method according to Claim 248, wherein the insulin polypeptide is insulin.

258. The method according to Claim 257, wherein the acyl oligomer is coupled to the lysine at the B29 position of the insulin.

259. The method according to Claim 248, wherein the insulin polypeptide-acyl oligomer conjugate is amphiphilically balanced.

260. The method according to Claim 248, wherein the acyl oligomer portion of the insulin polypeptide-acyl oligomer conjugate comprises a hydrophilic moiety and a lipophilic moiety.

261. The method according to Claim 260, wherein the hydrophilic moiety is a polyethylene glycol moiety.

262. The method according to Claim 261, wherein the polyethylene glycol moiety has between 1 and 50 polyethylene glycol subunits.

263. The method according to Claim 261, wherein the polyethylene glycol moiety has between 3 and 50 polyethylene glycol subunits.

264. The method according to Claim 261, wherein the polyethylene glycol moiety has between 2 and 10 polyethylene glycol subunits.

265. The method according to Claim 261, wherein the polyethylene glycol moiety has between 4 and 10 polyethylene glycol subunits.

266. The method according to Claim 261, wherein the polyethylene glycol moiety has at least 2 polyethylene glycol subunits.

267. The method according to Claim 260, wherein the lipophilic moiety is an alkyl or a fatty acid moiety.

268. The method according to Claim 267, wherein the lipophilic moiety has between 1 and 28 carbon atoms.

269. The method according to Claim 267, wherein the lipophilic moiety has between 2 and 24 carbon atoms.

270. The method according to Claim 267, wherein the lipophilic moiety has between 3 and 18 carbon atoms.

271. The method according to Claim 267, wherein the lipophilic moiety has between 4 and 12 carbon atoms.

272. The method according to Claim 267, wherein the lipophilic moiety has between 5 and 7 carbon atoms.

273. The method according to Claim 267, wherein the lipophilic moiety has between 4 and 14 carbon atoms.

274. The method according to Claim 248, wherein the enzymatically cleaving of the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-oligomer conjugate.

275. The method according to Claim 274, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

276. The method according to Claim 249, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the enzymatically cleaving of the connecting peptide from the proinsulin-acyl oligomer conjugate comprises:

contacting the proinsulin polypeptide-acyl oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-acyl oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin-acyl oligomer conjugate.

277. The method according to Claim 276, wherein the terminal amino acid residue is an arginine residue.

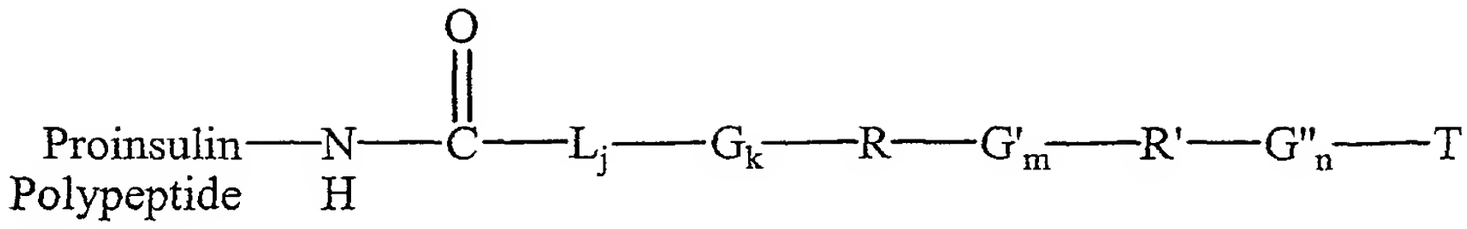
278. The method according to Claim 277, wherein the insulin polypeptide is insulin, and wherein the connecting peptide is C-human peptide.

279. The method according to Claim 276, wherein the contacting of the proinsulin-oligomer conjugate with a first enzyme and the contacting of the terminal amino acid residue-insulin polypeptide-acyl oligomer conjugate with a second enzyme occur substantially concurrently.

280. The method according to Claim 276, wherein the first enzyme and the second enzyme are provided in a mixture comprising the first enzyme and the second enzyme.

281. The method according to Claim 276, wherein the first enzyme is trypsin, and wherein the second enzyme is carboxy peptidase B.

282. A method of synthesizing an insulin polypeptide-acyl oligomer conjugate comprising cleaving one or more peptides from a proinsulin polypeptide-acyl oligomer conjugate comprising the following structure:



wherein:

L is a linker moiety;

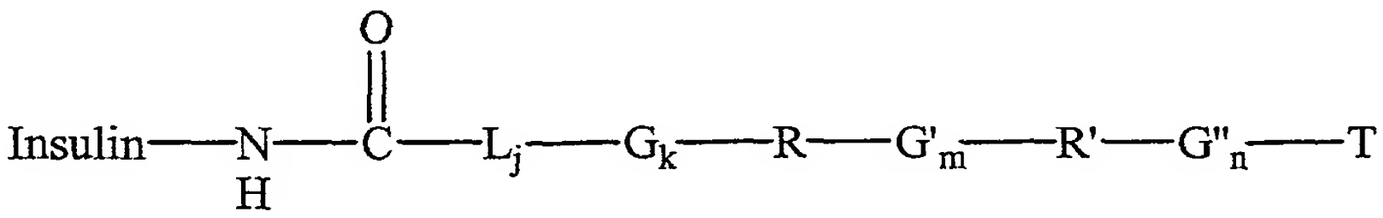
G, G' and G" are each spacer moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and

j, k, m and n are individually 0 or 1;

to provide the insulin polypeptide-acyl oligomer conjugate comprising the following structure:



283. The method according to Claim 282, wherein L is selected from the group consisting of alkyl moieties and fatty acid moieties.

284. The method according to Claim 282, wherein G, G' and G are individually selected from the group consisting of sugar, cholesterol, and glycerine moieties.

285. The method according to Claim 282, wherein T is selected from the group consisting of alkyl and alkoxy.

286. The method according to Claim 282, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

287. The method according to Claim 283, wherein the connecting peptide is a C-peptide polypeptide.

288. The method according to Claim 283, wherein the connecting peptide is C-peptide.

289. The method according to Claim 283, wherein the connecting peptide is devoid of lysine residues.

290. The method according to Claim 283, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

291. The method according to Claim 283, wherein the leader peptide is devoid of lysine residues.

292. The method according to Claim 282, wherein the proinsulin polypeptide is proinsulin.

293. The method according to Claim 282, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable.

294. The method according to Claim 282, wherein the insulin polypeptide is insulin.

295. The method according to Claim 294, wherein the oligomer is coupled to the lysine at the B29 position of the insulin.

296. The method according to Claim 282, wherein the insulin polypeptide-oligomer conjugate is amphiphilically balanced.

297. The method according to Claim 282, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

298. The method according to Claim 297, wherein the polyethylene glycol moiety has between 1 and 50 polyethylene glycol subunits.

299. The method according to Claim 297, wherein the polyethylene glycol moiety has between 3 and 50 polyethylene glycol subunits.

300. The method according to Claim 297, wherein the polyethylene glycol moiety has between 2 and 10 polyethylene glycol subunits.

301. The method according to Claim 297, wherein the polyethylene glycol moiety has between 4 and 10 polyethylene glycol subunits.

302. The method according to Claim 297, wherein the polyethylene glycol moiety has at least 2 polyethylene glycol subunits.

303. The method according to Claim 282, wherein the lipophilic moiety is an alkyl or a fatty acid moiety.

304. The method according to Claim 303, wherein the lipophilic moiety has between 1 and 28 carbon atoms.

305. The method according to Claim 303, wherein the lipophilic moiety has between 2 and 24 carbon atoms.

306. The method according to Claim 303, wherein the lipophilic moiety has between 3 and 18 carbon atoms.

307. The method according to Claim 303, wherein the lipophilic moiety has between 4 and 12 carbon atoms.

308. The method according to Claim 303, wherein the lipophilic moiety has between 5 and 7 carbon atoms.

309. The method according to Claim 303, wherein the lipophilic moiety has between 4 and 14 carbon atoms.

310. The method according to Claim 282, wherein:

R is an alkyl moiety having between 3 and 8 carbon atoms;

R' is polyethylene glycol having between 4 and 10 polyethylene glycol subunits;

T is lower alkyl or lower alkoxy; and

j, k, m and n are 0.

311. The method according to Claim 282, wherein:

R is an alkyl moiety having between 3 and 8 carbon atoms;

R' is polyethylene glycol having 7 polyethylene glycol subunits;

T is methoxy; and

j, k, m and n are 0.

312. The method according to Claim 282, wherein the enzymatically cleaving of the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate.

313. The method according to Claim 312, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

314. The method according to Claim 283, wherein the connecting peptide has a terminal amino acid residue at the first end, and wherein the enzymatically cleaving of the connecting peptide from the proinsulin polypeptide-acyl oligomer conjugate comprises:

contacting the proinsulin polypeptide-acyl oligomer conjugate with a first enzyme under conditions sufficient to provide a terminal amino acid residue-insulin polypeptide-acyl oligomer conjugate; and

contacting the terminal amino acid residue-insulin polypeptide-acyl oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin polypeptide-acyl oligomer conjugate.

315. The method according to Claim 314, wherein the terminal amino acid residue is an arginine residue.

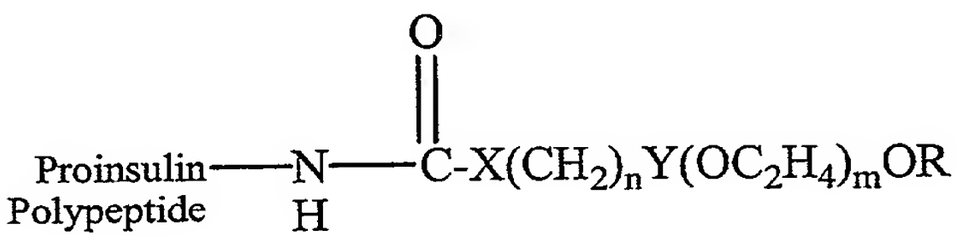
316. The method according to Claim 315, wherein the insulin polypeptide is insulin, and wherein the connecting peptide is human C-peptide.

317. The method according to Claim 314, wherein the contacting of the proinsulin-oligomer conjugate with a first enzyme and the contacting of the terminal amino acid residue-insulin polypeptide-oligomer conjugate with a second enzyme occur substantially concurrently.

318. The method according to Claim 317, wherein the first enzyme and the second enzyme are provided in a mixture comprising the first enzyme and the second enzyme.

319. The method according to Claim 315, wherein the first enzyme is trypsin, and wherein the second enzyme is carboxy peptidase B.

320. A method of synthesizing an insulin polypeptide-acyl oligomer conjugate comprising enzymatically cleaving one or more peptides from a proinsulin polypeptide-acyl oligomer conjugate comprising the following structure:



wherein:

X is an oxygen atom or a covalent bond;

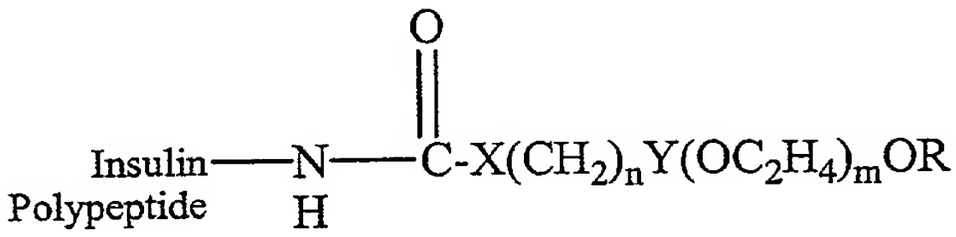
Y is an ester, an ether, a carbamate, a carbonate, or an amide bonding moiety;

n is between 1 and 30;

m is between 1 and 50; and

R is alkyl;

to provide the insulin polypeptide-acyl oligomer conjugate comprising the structure of:



321. The method according to Claim 320, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

322. The method according to Claim 321, wherein the connecting peptide is a C-peptide polypeptide.

323. The method according to Claim 321, wherein the connecting peptide is C-peptide.

324. The method according to Claim 321, wherein the connecting peptide is devoid of lysine residues.

325. The method according to Claim 321, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

326. The method according to Claim 325, wherein the leader peptide is devoid of lysine residues.

327. The method according to Claim 320, wherein the proinsulin polypeptide is proinsulin.

328. The method according to Claim 320, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable.

329. The method according to Claim 320, wherein the insulin polypeptide is insulin.

330. The method according to Claim 329, wherein the acyl oligomer is coupled to the lysine at the B29 position of the insulin.

331. The method according to Claim 320, wherein the insulin polypeptide-acyl oligomer conjugate is amphiphilically balanced.

332. The method according to Claim 320, wherein m is between 3 and 16.

333. The method according to Claim 320, wherein m is between 4 and 14.

334. The method according to Claim 320, wherein m is between 5 and 10.

335. The method according to Claim 320, wherein n is between 3 and 18.

336. The method according to Claim 320, wherein n is between 4 and 14.

337. The method according to Claim 320, wherein n is between 5 and 10.

338. The method according to Claim 320, wherein R is lower alkyl.

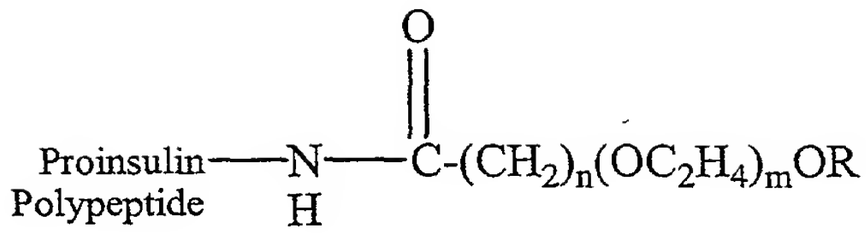
339. The method according to Claim 320, wherein R is C_1 to C_3 alkyl.

340. The method according to Claim 320, wherein R is methyl.

341. The method according to Claim 320, wherein the enzymatically cleaving of the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate.

342. The method according to Claim 341, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

343. A method of synthesizing an insulin polypeptide-acyl oligomer conjugate comprising enzymatically cleaving one or more peptides from a proinsulin polypeptide-acyl oligomer conjugate comprising the following structure:



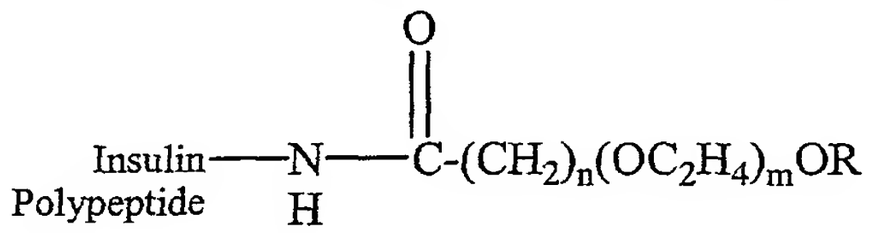
wherein:

n is between 1 and 30;

m is between 1 and 50; and

R is alkyl;

to provide the insulin polypeptide-acyl oligomer conjugate comprising the structure of:



344. The method according to Claim 343, wherein the insulin polypeptide has an A-chain polypeptide and a B-chain polypeptide, and wherein the one or more peptides comprise a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

345. The method according to Claim 344, wherein the connecting peptide is a C-peptide polypeptide.

346. The method according to Claim 344, wherein the connecting peptide is C-peptide.

347. The method according to Claim 344, wherein the connecting peptide is devoid of lysine residues.

348. The method according to Claim 344, wherein the one or more peptides further comprise a leader peptide coupled to the N-terminus of the B-chain polypeptide.

349. The method according to Claim 348, wherein the leader peptide is devoid of lysine residues.

350. The method according to Claim 343, wherein the proinsulin polypeptide is proinsulin.

351. The method according to Claim 343, wherein the proinsulin polypeptide is proinsulin coupled at the N-terminus of the B-chain to a leader peptide by a peptide bond that is cleavable.

352. The method according to Claim 343, wherein the insulin polypeptide is insulin.

353. The method according to Claim 352, wherein the acyl oligomer is coupled to the lysine at the B29 position of the insulin.

354. The method according to Claim 343, wherein the insulin polypeptide-acyl oligomer conjugate is amphiphilically balanced.

355. The method according to Claim 343, wherein m is between 3 and 16.

356. The method according to Claim 343, wherein m is between 4 and 14.

357. The method according to Claim 343, wherein m is between 5 and 10.

358. The method according to Claim 343, wherein n is between 3 and 18.

359. The method according to Claim 343, wherein n is between 4 and 14.

360. The method according to Claim 343, wherein n is between 5 and 10.

361. The method according to Claim 343, wherein R is lower alkyl.

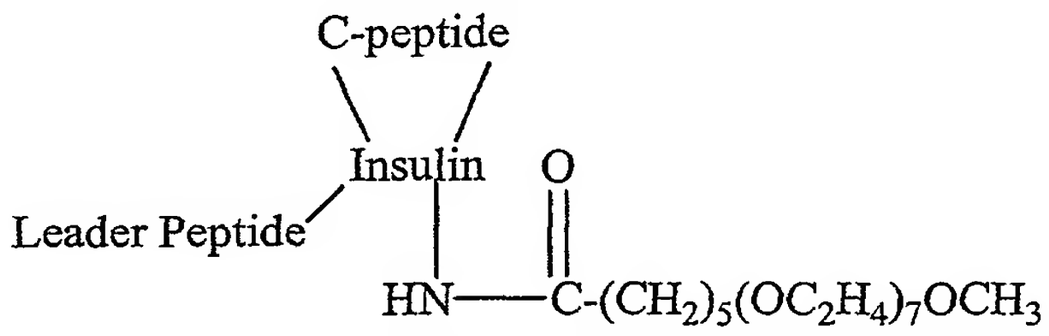
362. The method according to Claim 343, wherein R is C_1 to C_3 alkyl.

363. The method according to Claim 343, wherein R is methyl.

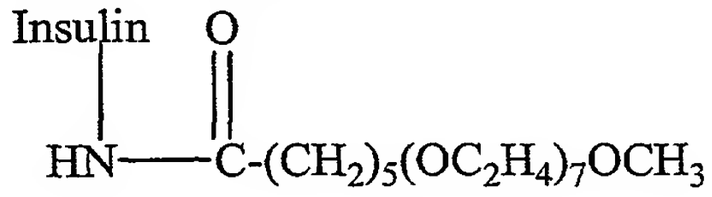
364. The method according to Claim 343, wherein the enzymatically cleaving of the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate comprises contacting the proinsulin polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the insulin polypeptide under conditions sufficient to cleave the one or more peptides from the proinsulin polypeptide-acyl oligomer conjugate.

365. The method according to Claim 364, wherein the one or more enzymes are selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.

366. A method of synthesizing an insulin-acyl oligomer conjugate comprising enzymatically cleaving a leader peptide and a C-peptide from a proinsulin polypeptide-acyl oligomer conjugate comprising the following structure:



to provide the insulin-acyl oligomer conjugate comprising the following structure:



367. The method according to Claim 366, wherein the leader peptide is devoid of lysine residues.

368. The method according to Claim 366, wherein the enzymatically cleaving of the C-peptide and the leader peptide from the proinsulin polypeptide-acyl oligomer conjugate comprises:

contacting the proinsulin polypeptide-oligomer conjugate with a first enzyme under conditions sufficient to provide a (Arg^{31})-insulin-oligomer conjugate; and

contacting the (Arg^{31})-insulin polypeptide-oligomer conjugate with a second enzyme under conditions sufficient to provide the insulin polypeptide-oligomer conjugate.

369. The method according to Claim 368, wherein the first enzyme is trypsin.

370. The method according to Claim 368, wherein the second enzyme is carboxy peptidase B.

371. A method of synthesizing a proinsulin polypeptide-oligomer conjugate comprising contacting a proinsulin polypeptide with an oligomer comprising a hydrophilic moiety and a lipophilic moiety under conditions sufficient to provide the proinsulin polypeptide-oligomer conjugate.

372. The method according to Claim 371, wherein the proinsulin polypeptide comprises an insulin polypeptide having an A-chain polypeptide, a B-chain polypeptide, and a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide.

373. The method according to Claim 372, wherein the connecting peptide is a C-peptide polypeptide.

374. The method according to Claim 372, wherein the connecting peptide is C-peptide.

375. The method according to Claim 372, wherein the connecting peptide is devoid of lysine residues.

376. The method according to Claim 372, wherein the proinsulin polypeptide further comprises a leader peptide coupled to the N-terminus of the B-chain polypeptide.

377. The method according to Claim 376, wherein the leader peptide is devoid of lysine residues.

378. The method according to Claim 371, wherein the proinsulin polypeptide comprises an insulin polypeptide having an A-chain polypeptide and a B-chain polypeptide, a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and at

a second end to the N-terminus of the A-chain polypeptide, and a leader peptide coupled to the N-terminus of the B-chain polypeptide.

379. The method according to Claim 378, wherein the insulin polypeptide is insulin.

380. The method according to Claim 379, wherein the oligomer is coupled to the lysine at the B29 position of the insulin.

381. The method according to Claim 371, wherein the proinsulin polypeptide is proinsulin.

382. The method according to Claim 371, wherein the insulin polypeptide-oligomer conjugate is amphiphilically balanced.

383. The method according to Claim 371, wherein the oligomer is present as a substantially monodispersed mixture.

384. The method according to Claim 371, wherein the oligomer is present as a monodispersed mixture.

385. The method according to Claim 371, wherein the hydrophilic moiety is a polyalkylene glycol moiety.

386. The method according to Claim 385, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

387. The method according to Claim 385, wherein the polyalkylene glycol moiety has between 1 and 50 polyalkylene glycol subunits.

388. The method according to Claim 385, wherein the polyalkylene glycol moiety has between 3 and 50 polyalkylene glycol subunits.

389. The method according to Claim 385, wherein the polyalkylene glycol moiety has between 2 and 10 polyalkylene glycol subunits.

390. The method according to Claim 385, wherein the polyalkylene glycol moiety has between 4 and 10 polyalkylene glycol subunits.

391. The method according to Claim 385, wherein the polyalkylene glycol moiety has at least 2 polyalkylene glycol subunits.

392. The method according to Claim 371, wherein the lipophilic moiety is an alkyl or fatty acid moiety.

393. The method according to Claim 371, wherein the lipophilic moiety has between 1 and 28 carbon atoms.

394. The method according to Claim 371, wherein the lipophilic moiety has between 2 and 24 carbon atoms.

395. The method according to Claim 371, wherein the lipophilic moiety has between 3 and 18 carbon atoms.

396. The method according to Claim 371, wherein the lipophilic moiety has between 4 and 12 carbon atoms.

397. The method according to Claim 371, wherein the lipophilic moiety has between 5 and 7 carbon atoms.

398. The method according to Claim 371, wherein the lipophilic moiety has between 4 and 14 carbon atoms.

399. The method according to Claim 371, wherein the oligomer comprises the structure of Formula VI:

$$A\text{——}L_j\text{——}G_k\text{——}R\text{——}G'_m\text{——}R'\text{——}G''_n\text{——}T \qquad \text{(VI)}$$

wherein:

A is an activatable moiety selected from the group consisting of -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, and NH_2;

L is a linker moiety selected from the group consisting of alkyl moieties and fatty acid moieties;

G, G' and G" are each spacer moieties individually selected from the group consisting of sugar, cholesterol, and glycerine moieties;

R is a lipophilic moiety and R' is a polyalkylene glycol moiety, or R' is the lipophilic moiety and R is the polyalkylene glycol moiety;

T is a terminating moiety; and

j, k, m and n are individually 0 or 1.

400. The method according to Claim 399, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

401. The method according to Claim 400, wherein the polyethylene glycol moiety has between 1 and 50 polyethylene glycol subunits.

402. The method according to Claim 400, wherein the polyethylene glycol moiety has between 3 and 50 polyethylene glycol subunits.

403. The method according to Claim 400, wherein the polyethylene glycol moiety has between 2 and 10 polyethylene glycol subunits.

404. The method according to Claim 400, wherein the polyethylene glycol moiety has between 4 and 10 polyethylene glycol subunits.

405. The method according to Claim 400, wherein the polyethylene glycol moiety has at least 2 polyethylene glycol subunits.

406. The method according to Claim 399, wherein the lipophilic moiety is an alkyl or a fatty acid moiety.

407. The method according to Claim 399, wherein the lipophilic moiety has between 1 and 28 carbon atoms.

408. The method according to Claim 399, wherein the lipophilic moiety has between 2 and 24 carbon atoms.

409. The method according to Claim 399, wherein the lipophilic moiety has between 3 and 18 carbon atoms.

410. The method according to Claim 399, wherein the lipophilic moiety has between 4 and 12 carbon atoms.

411. The method according to Claim 399, wherein the lipophilic moiety has between 5 and 7 carbon atoms.

412. The method according to Claim 399, wherein the lipophilic moiety has between 4 and 14 carbon atoms.

413. The method according to Claim 399, wherein:

A is a carboxylic acid moiety;

R is an alkyl moiety having between 3 and 8 carbon atoms;

R' is polyethylene glycol having between 4 and 10 polyethylene glycol subunits;

T is lower alkyl or lower alkoxy; and

j, k, m and n are 0.

414. The method according to Claim 399, wherein:

A is a carboxylic acid moiety;

R is an alkyl moiety having between 3 and 8 carbon atoms;

R' is polyethylene glycol having 7 polyethylene glycol subunits;

T is methoxy; and

j, k, m and n are 0.

415. The method according to Claim 371, wherein the oligomer comprises the structure of Formula VII:

$$A\text{-}X(CH_2)_mY(OC_2H_4)_nOR \qquad (VII)$$

wherein:

A is an activatable moiety selected from the group consisting of -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, and NH_2;

X is an oxygen atom or a covalent bond, with the provisio that X is not an oxygen atom when A is -OH;

Y is an ester, an ether, a carbamate, a carbonate, or an amide bonding moiety;

m is between 1 and 25;

n is between 1 and 25;

and R is alkyl.

416. The method according to Claim 415, wherein m is between 3 and 16.

417. The method according to Claim 415, wherein m is between 4 and 14.

418. The method according to Claim 415, wherein m is between 5 and 10.

419. The method according to Claim 415, wherein n is between 3 and 18.

420. The method according to Claim 415, wherein n is between 4 and 14.

421. The method according to Claim 415, wherein n is between 5 and 10.

422. The method according to Claim 415, wherein R is lower alkyl.

423. The method according to Claim 415, wherein R is C_1 to C_3 alkyl.

424. The method according to Claim 415, wherein R is methyl.

425. The method according to Claim 371, wherein the oligomer comprises the structure of Formula VIII:

$$A\text{-}(CH_2)_m(OC_2H_4)_nOR \qquad (VIII)$$

wherein:

A is an activatable moiety selected from the group consisting of -C(O)-OH, C(S)-OH, -C(S)-SH, -OH, -SH, and NH_2;

m is between 1 and 25;

n is between 1 and 25; and

R is alkyl.

426. The method according to Claim 425, wherein m is between 3 and 16.

427. The method according to Claim 425, wherein m is between 4 and 14.

428. The method according to Claim 425, wherein m is between 5 and 10.

429. The method according to Claim 425, wherein n is between 3 and 18.

430. The method according to Claim 425, wherein n is between 4 and 14.

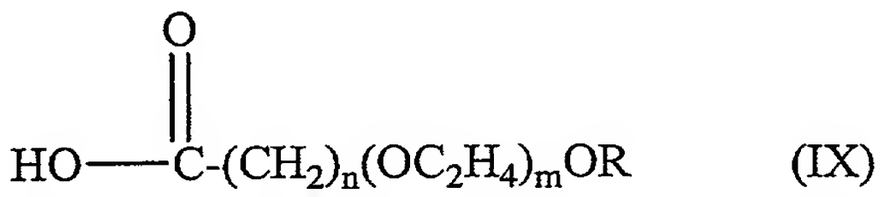
431. The method according to Claim 425, wherein n is between 5 and 10.

432. The method according to Claim 425, wherein R is lower alkyl.

433. The method according to Claim 425, wherein R is C_1 to C_3 alkyl.

434. The method according to Claim 425, wherein R is methyl.

435. The method according to Claim 371, wherein the oligomer comprises the structure of Formula IX:



wherein m is between 1 and 25, n is between 1 and 25, and R is alkyl.

436. The method according to Claim 435, wherein m is between 3 and 16.

437. The method according to Claim 435, wherein m is between 4 and 14.

438. The method according to Claim 435, wherein m is between 5 and 10.

439. The method according to Claim 435, wherein n is between 3 and 18.

440. The method according to Claim 435, wherein n is between 4 and 14.

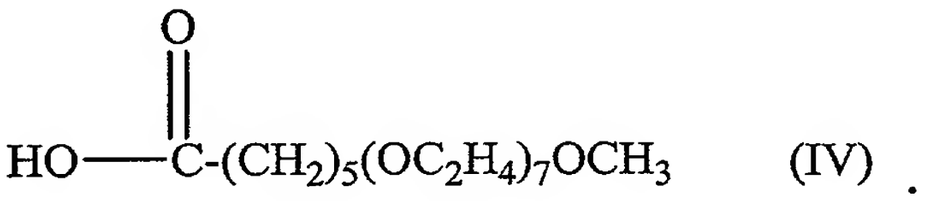
441. The method according to Claim 435, wherein n is between 5 and 10.

442. The method according to Claim 435, wherein R is lower alkyl.

443. The method according to Claim 435, wherein R is C_1 to C_3 alkyl.

444. The method according to Claim 435, wherein R is methyl.

445. The method according to Claim 371, wherein the oligomer comprises the structure of Formula IV:



(IV)

446. A proinsulin polypeptide-oligomer conjugate comprising:

a proinsulin polypeptide comprising an insulin polypeptide; and

an oligomer comprising a hydrophilic moiety and a lipophilic moiety coupled to the insulin polypeptide portion of the proinsulin polypeptide.

447. The conjugate according to Claim 446, wherein the proinsulin polypeptide comprises an insulin polypeptide having an A-chain polypeptide and a B-chain polypeptide; a connecting peptide coupled at a first end to the C-terminus of the B-chain polypeptide and coupled at a second end to the N-terminus of the A-chain polypeptide; and a leader peptide coupled to the N-terminus of the B-chain polypeptide.

448. The conjugate according to Claim 447, wherein the connecting peptide is C-peptide.

449. The conjugate according to Claim 447, wherein the connecting peptide is devoid of lysine residues.

450. The conjugate according to Claim 446, wherein the proinsulin polypeptide is proinsulin.

451. The conjugate according to Claim 446, wherein the insulin polypeptide is insulin.

452. The conjugate according to Claim 446, wherein the oligomer is coupled to the insulin polypeptide portion of the proinsulin polypeptide at a lysine residue of the insulin polypeptide.

453. The conjugate according to Claim 452, wherein the insulin polypeptide is insulin and wherein the lysine residue is at the B29 position of the insulin.

454. The conjugate according to Claim 446, wherein the hydrophilic moiety is a polyalkylene glycol moiety.

455. The conjugate according to Claim 454, wherein the polyalkylene glycol moiety is a polyethylene glycol moiety.

456. The conjugate according to Claim 454, wherein the polyalkylene glycol moiety has between 4 and 10 polyalkylene glycol subunits.

457. The conjugate according to Claim 446, wherein the lipophilic moiety is an alkyl or fatty acid moiety.

458. The conjugate according to Claim 457, wherein the lipophilic moiety has between 3 and 8 carbon atoms.

459. A method of synthesizing a C-peptide polypeptide-oligomer conjugate comprising:

contacting a pro-C-peptide polypeptide comprising a C-peptide polypeptide coupled to one or more peptides by peptide bond(s) that are cleavable to yield the C-peptide polypeptide with an oligomer under conditions sufficient to couple the oligomer to the C-peptide polypeptide portion of the pro-C-peptide polypeptide and provide a pro-C-peptide polypeptide-oligomer conjugate; and

cleaving the one or more peptides from the pro-C-peptide polypeptide-oligomer conjugate to provide the C-peptide polypeptide-oligomer conjugate.

460. The method according to Claim 459, wherein the C-peptide polypeptide is C-peptide.

461. The method according to Claim 459, wherein the pro-C-peptide polypeptide is a proinsulin polypeptide.

462. The method according to Claim 459, wherein the pro-C-peptide polypeptide is proinsulin.

463. The method according to Claim 459, wherein the one or more peptides is an insulin polypeptide.

464. The method according to Claim 459, wherein the one or more peptides is insulin.

465. The method according to Claim 459, wherein the oligomer comprises a hydrophilic moiety and a lipophilic moiety.

466. The method according to Claim 459, wherein the cleaving of the one or more peptides from the pro-C-peptide polypeptide-oligomer conjugate comprises contacting the pro-C-peptide polypeptide-oligomer conjugate with one or more enzymes that are capable of cleaving the bond(s) between the one or more peptides and the C-peptide polypeptide under conditions sufficient to cleave the one or more peptides from the pro-C-peptide polypeptide-oligomer conjugate.

467. The method according to Claim 466, wherein the one or more enzymes is selected from the group consisting of trypsin, carboxy peptidase B, and mixtures thereof.